IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of	:
HISAAKI CHAKI et al.	:GROUP ART UNIT: 1621
Serial NO. 09/830,559	:EXAMINER: Peter G O'Sullivan
Filed: May 7, 2001	:
For: NOVEL COMPOUNDS AND	:
PHARMACEUTICAL USE THEREOF	:

D E C L A R A T I O N

Honorable Commissioner of Patents and Trademarks Washington, D.C. 20231

Sir:

I, Yukihiko Aikawa, a Japanese citizen, residing at 230, Mizuhashinakamura-Machi, Toyama-shi, Toyama-ken, Japan, declare:

That I am one of the inventors of the above-identified application;

That I am a graduate of Toyama Medical and Pharmaceutical University, from which I received a Master's degree in 1987, and have, since April 1987, been employed by Toyama Chemical Co., Ltd. and have been engaged in research on the development of drugs and medicine in the Department of Chemical Research of said company, and I received a degree of Doctor of Pharmaceutical Sciences from Toyama Medical and Pharmaceutical University in March, 2002

That I have read and understood the office action dated March 25, 2004 and September 24, 2004, and the advisory action dated December 3, 2004;

That I conducted the following comparative experiments to compare the effects of the following typical compounds (a), (b) and (c) of the present application:

(a) 3-[5-(2,4-diisobutoxybenzoyl)-2-isobutoxyphenyl]propanoic acid

(b)3-[5-(4-isobutoxybenzoyl)-2-isobutoxyphenyl]propanoic acid

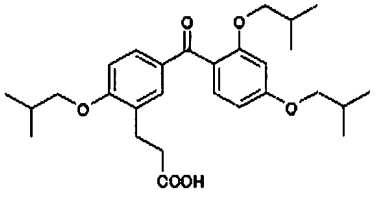
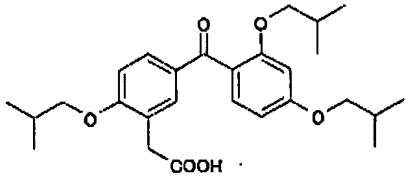
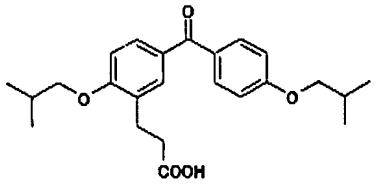
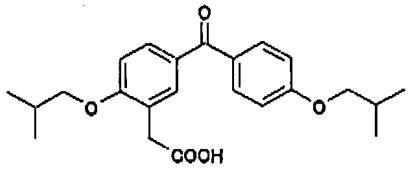
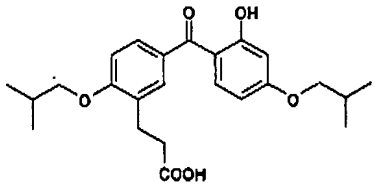
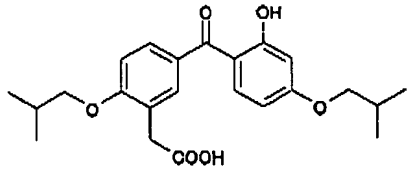
(c)3-[5-(2-hydroxy-4-isobutoxybenzoyl)-2-isobutoxyphenyl]propanoic acid

with the effects of the following compound (d), (e) and (f) of the present application:

(d)2-[5-(2,4-diisobutoxybenzoyl)-2-isobutoxyphenyl]acetic acid

(e)2-[5-(4-isobutoxybenzoyl)-2-isobutoxyphenyl]acetic acid

(f)2-[5-(2-hydroxy-4-isobutoxybenzoyl)-2-isobutoxyphenyl]acetic acid

Present application	Comparative compounds
(a)  Example No. 12	(d)  Example No. 7
(b)  Example No. 25(8)	(e)  Example No. 25(1)
(c)  Example No. 38	(f)  Example No. 39(5)

I. Synthesis of the compounds used in the comparative experiments

(1) Compound (a), (b), (d) and (e) were synthesized in the same manner as in the Example 7 of the present specification. The physical properties of the compounds were identical with those stated in

the present specification.

(2) Compound (c) and (f) were synthesized in the same manner as in the Example 38 of the present specification. The physical properties of the compounds were identical with those stated in the present specification.

II. pharmacological activities of the test compounds

(1) Test compounds

Compound (a),(b),(c),(d),(e) and (f), which were synthesized in research laboratory in Toyama Chemical Co., Ltd., were used for the experiments.

(2) Testing method

Activities on AP-1 binding reaction to recognition sequence (ELISA)

The test of ELISA was carried out in accordance with the same manner as in the Test Example 1 of the present specification.

Test Example 1:

Nuclear extract protein containing transcription factor AP-1 prepared from HeLa cells was coated on 96-well ELISA plate (100 ng/well) in Hepes buffer (20 mM Hepes-potassium hydroxide (pH 7.9), 0,5 mM ethylenediamine-tetraacetic acid, 50 mM potassium chloride, 10% glycerol). After washing, a blocking treatment was carried out with bovine serum albumin, and then used for a binding assay using nuclear extract protein.

On the other hand, Jun peptide and N-terminal biotinylated tetraglycine Fos peptide containing a DNA-binding site [Nature, Vol. 373, Pages 257-261, 1995] were synthesized and separately dissolved in tris buffer (20 mM tris-hydrochloride (pH 7.5), 50 mM potassium

chloride, 1 mM ethylenediaminetetraacetic acid, 10 mM magnesium chloride, 1 mM dithiothreitol, 0.5M guanidine hydrochloride, 30% glycerol). Equimolar quantities of both the solutions were mixed together, and the mixture was used as an AP-1 complex (Fos/Jun peptide). The AP-1 complex was added to avidin-coating 96-well ELISA plate (10 pmol/well), washed, and then blocked with bovine serum albumin. The product was used for binding assay using AP-1 complex.

On the basis of the above-mentioned two coated AP-1, a digoxigenin-labeled double stranded oligonucleotide (22-mer) containing an AP-1 binding sequence (3'-TGAGTCA-5') which has been synthesized elsewhere was reacted in the presence and absence of a sample at room temperature for 30-60 minutes in a binding reaction solution [Hepes buffer or 25 mM tris-hydrochloric acid (pH 7.9), 0.5 mM ethylenediaminetetraacetic acid, 0.05% Nonidet P-40, 10% glycerol]. After of the reaction, unbound labeled oligonucleotide was washed out with Hepes buffer solution containing 0.05% of Tween-20. Then, an anti-digoxigenin antibody labeled with peroxidase was added, and reacted with the labeled oligonucleotide bound to AP-1. After washing out the excessive antibody with Hepes buffer containing 0.05% of Tween-20, the residue was reacted for a predetermined period of time in a 100 mM citrate buffer (pH 5.0) containing hydrogen peroxide by using o-phenylenediamine as a substrate. After adding sulfuric acid solution to each well, absorbance (492 nm) was measured. Taking the absorbance in the absence of test compound as 100%, inhibition rate of test compound was calculated from the absorbance in the presence of test compound.

The results are shown in Table 1.

Table 1

Compound	Inhibition rate % (500 μM)
(a)	90
(d)	46
(b)	79
(e)	17
(c)	91
(f)	58

The undersigned declarant declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 6th day of January, 2005.

Yukihiko Aikawa

Yukihiko AIKAWA